PATENT
Docket No. 308072000110
Client Ref. ARC 088 DIV 1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the application of:

David W. SCOTT and
Elias T. ZAMBIDIS

Examiner: M. Wilson

Group Art Unit: 1633

Serial No.: 09/160,076

Filing Date: September 24 1998

For: TOLEROGENIC FUSION PROTEINS OF IMMUNOGLOBULINS AND METHODS FOR INDUCING AND MAINTAINING TOLERANCE

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DECLARATION UNDER 37 C.F.R. § 1.132

I, David W. Scott, one of the co-inventors of the invention described in the above identified patent application, declare and affirm that:

1. I am currently Head of the Immunology Department at the Jerome Holland Laboratories of the American Red Cross, Rockville, Maryland. I am also Professor and Chair of the Department of Immunology at the George Washington University Medical Center. I have been actively involved in the study of basic immunology, with particular emphasis on immunological tolerance, since 1966. I have published numerous research papers, review articles and book chapters on this subject. A curriculum vitae, including a selected list of publications, is attached hereto as Exhibit A.

2. I am an inventor on the above-referenced patent application. In addition to the experimental data presented in the specification, additional experiments have been performed in accordance with the teachings of the specification, using a variety of different antigenic polypeptides in fusion proteins. These experiments (described in detail below) have demonstrated that a pharmaceutical composition comprising a pharmaceutically acceptable excipient and non-tumor lymphoid or hematopoietic cells

comprising an IgG fusion protein allow for induction of tolerance to many different antigens. These experiments also demonstrate that tolerance can be induced using either an entire antigenic polypeptide or portion thereof in the fusion protein (as addressed in the First Declaration of Dr. David W. Scott, filed September 21, 2001).

3. In addition to the lymphoid cell comprising a fusion protein comprising residues 12-26 of the bacteriophage λ c1 protein described in Examples I-V of the instant specification (at pages 31-43), non-tumor lymphoid or hematopoietic cells comprising the following six antigenic polypeptides (or portion thereof) have been prepared and studied:

(i) myelin basic protein - full-length protein;
(ii) glutamic acid decarboxylase (GAD65) - full-length protein;
(iii) insulin B chain - residues 9-23;
(iv) interreceptor retinal binding protein (IRBP) - residues 161-180;
(v) bacteriophage λ c1 protein - full-length protein; and
(vi) ovalbumin - full-length protein.

Each of these antigenic polypeptides, or portion thereof, was incorporated into an IgG fusion protein according to the teachings of the specification and the IgG fusion proteins were expressed on lymphoid or haemopoietic cells, also in accordance with the teachings of the specification. Pharmaceutical compositions comprising (a) non-tumor lymphoid or hematopoietic cells, specifically B cells, expressing the IgG fusion proteins; and (b) a pharmaceutically acceptable excipient, were administered to a mammalian host, and tolerance induction was studied. As described in further detail below, tolerance was induced using non-tumor lymphoid or hematopoietic cells comprising fusion protein comprising an immunoglobulin heavy or light chain and each of the six antigenic polypeptides or portion thereof, listed above, thereby demonstrating the general applicability of the pharmaceutical compositions of the invention in inducing tolerance to a wide variety of different antigens, even when the major epitopes for a given MHC haplotype are not known. Some of this work has recently been published in a peer-reviewed journal *J. Immunology*. *See* Melo et al., (2002) *J. Immunol.* 168:4788-95.

4. For the study of myelin basic protein (MBP), the experimental allergic encephalitis (EAE) mouse model was used. The EAE model is a well established, art-recognized system for studying the autoimmune reaction to myelin basic protein, MBP, as a model for multiple sclerosis. An MBP-IgG fusion retroviral vector construct was prepared using the full-length MBP nucleotide sequence in the construct. Non-tumor lymphoid or hematopoietic cells, specifically, splenic B cell blasts, were infected with the retrovirus encoding the IgG fusion protein. An ovalbumin-IgG (OVA-IgG) fusion construct was used as a control. After expansion of the cells, they were suspended in a pharmaceutically acceptable excipient (PBS), and the pharmaceutical composition of cells and excipient was injected intravenously into syngeneic recipients after passive transfer of MBP-specific T cells into the recipients. Mice were followed daily for signs of paralysis (as an indicator of an immune response to MBP) until moribund/sacrifice and their average EAE score (on a scale of 1-5) was assessed. As shown in Figure 1, attached hereto as Exhibit B, mice that were treated with the control composition, OVA-IgG expressing cells and a pharmaceutically acceptable excipient, had EAE scores of at least 3 within first five days of treatment and reached EAE scores of 5 by day 10. In contrast, mice that were treated with the pharmaceutical composition comprising MBP-IgG expressing cells and a pharmaceutically acceptable excipient, had EAE scores of only 1-2 over the course of 20 days, indicating that the MBP-specific T cells that had been transferred into the recipients were effective in inducing tolerance.

5. For the study of glutamic acid decarboxylase (GAD) and insulin receptor B chain residues 9-23, the NOD mouse model was used. The NOD mouse model is a well established, art-recognized system for studying autoimmune diabetes. A GAD-IgG fusion retroviral vector construct and an insulin B chain 9-23 IgG fusion retroviral vector construct (B9-23-IgG) were prepared using a nucleotide sequence encoding the full-length GAD and a nucleotide sequence encoding residues 9-23 of insulin B chain, respectively, in the constructs. Splenic B cell blasts from NOD mice were infected with the retrovirus encoding either the GAD-IgG or B9-23-IgG fusion protein. A lambda repressor cI 1-102 immunoglobulin fusion protein (1-102-IgG) construct was used as a control. The transduced NOD B cell blasts were suspended in a pharmaceutically

acceptable excipient, and the pharmaceutical composition of cells and pharmaceutically acceptable excipient (PBS) were transferred to NOD recipients intraperitoneally at 10 weeks (by which time the NOD mice exhibit signs of early diabetes). Mice were followed weekly for glucose levels until moribund. Figure 2, attached hereto as Exhibit C, is a graph showing the percentage of mice exhibiting diabetes from 10 weeks to 19 weeks, wherein the mice were either untreated (squares), treated with the control composition comprising cells expressing the lambda cI 1-102-IgG construct (circles), treated with the composition comprising cell expressing the GAD-IgG construct (triangles), or treated with the composition comprising cells expressing the B9-23-IgG construct (diamonds). The results demonstrated that a lower percentage of mice treated with either the pharmaceutical composition comprising GAD-IgG-expressing cells or B9-23-IgG-expressing cells exhibited diabetes over the course of the experiment. Importantly, it was found that a single treatment with the composition comprising cell comprising the GAD-IgG construct led to a significant delay in the onset of diabetes, as measured by glucose levels and prolongation of life. In addition, a single treatment with a pharmaceutical composition comprising either GAD-IgG-expressing cells or insulin B9-23-IgG-expressing cells (and a pharmaceutically acceptable excipient) after clinical signs of diabetes (week 10) showed significant efficacy. Analysis of islet pathology in GAD-IgG or B9-23-IgG treated mice suggested that remaining islet survival was maintained, despite inflammation in surrounding areas. Importantly, these data show that NOD B cells can be tolerogenic antigen presenting cells (APC). Thus, these experiments demonstrate that the compositions comprising cell expressing GAD-IgG and B9-23-IgG (and a pharmaceutically acceptable excipient) were effective in inducing tolerance in an animal model of autoimmune diabetes.

6. For the study of interreceptor retinal binding protein (IRBP), the experimental autoimmune uveitis mouse model was used. The uveitis model is a well established model for studying the autoimmune reaction to IRBP. An IRBP-IgG fusion retroviral vector construct was prepared using the nucleotide sequence of residues 161-180 in the construct, since residues 161-180 had previously been established as a uveitogenic peptide. Silver et al., *Invest. Ophthalmol. Vis. Sci.* (1995), 36:946-954. Non-

tumor splenic B cell blasts were infected with the retrovirus encoding the IgG fusion protein, suspended in a pharmaceutically acceptable excipient (PBS), and the pharmaceutical composition comprising the cells and the pharmaceutically acceptable excipient used to treat unprimed mice challenged with p161-180 from human IRBP in complete Freund's adjuvant to induce uveitis. To test the efficacy of already immune animals, seven-day primed mice received a pharmaceutical composition comprising similarly transduced B cell blasts and a pharmaceutically acceptable excipient. The results of these experiments are described in detail in Agarwal, R.K. et al., *J. Clin. Invest.* (2000), 106:245-252, a copy of which is attached hereto as Exhibit D. The results demonstrated that a single infusion of a pharmaceutical composition comprising transduced cells and a pharmaceutically acceptable excipient, 10 days before uveitogenic challenge, protected mice from clinical disease induced with the epitope or with the native IRBP protein. Furthermore, the treatment was protective when initiated 7 days after uveitogenic immunization or concurrently with adoptive transfer of primed uveitogenic T cells. Thus, these experiments demonstrate that the pharmaceutical composition comprising lymphoid cells expressing the IRBP 161-180-IgG construct, and a pharmaceutically acceptable excipient, was effective in inducing tolerance in an animal model of autoimmune uveitis.

7. For the study of induction of hyporesponsiveness to intact foreign protein, an immunoglobulin fusion retroviral vector construct was prepared using the full-length nucleotide sequence of bacteriophage lambda repressor cI protein (encoding residues 1-102). Non-tumor bone marrow cells or peripheral B cells were infected with the retrovirus encoding the IgG fusion protein, the cells were suspended in a pharmaceutically acceptable excipient, and the pharmaceutical composition comprising the cells and excipient was administered to syngeneic mice to test for tolerance. The results of these experiments are described in detail in Kang et al., *Proc. Natl. Acad. Sci. USA* (1999), 96:8609-8614, a copy of which is attached hereto as Exhibit E. The results demonstrate that when the experimental mice were challenged with the p1-102 peptide, they failed to respond as effectively as mice treated with mock-transfected control cells to the major epitopes of p1-102 recognized by mice of the haplotypes used in the study.

The results of these experiments show that administration of a pharmaceutical composition comprising non-tumor cells expressing a full-length protein fused to IgG, and a pharmaceutically acceptable excipient, is an effective approach for the purpose of inducing tolerance to all known epitopes of an antigen.

8. In addition to the full-length lambda repressor cI protein described in paragraph 7, we have engineered a fusion protein construct that contains a full-length ovalbumin protein. Ovalbumin is an allergenic chicken protein, and is an art-recognized model antigen. Cells transduced with this OVA-IgG construct were effective in inducing tolerance to ovalbumin (data not shown), similar to the results for the other experimental systems described herein.

9. I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the above-identified application or any patent issuing thereon.

Executed this 19th day of June, ~~2001~~ 2002

David W. Scott

EXHIBIT A

BIOGRAPHICAL SKETCH

Give the following information for th key personnel and consultants and collaborators. Begin with the principal investigator/program director. Photocopy this page for each person.

NAME	POSITION TITLE
David W. Scott	Head, Department of Immunology

EDUCATION *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED	FIELD OF STUDY
Antioch College, Yellow Springs, OH	Left after 3	years w/o degree to begin grad work	
University of Chicago, Chicago, IL	M.S.	1964	Microbiology
Yale University, New Haven, CT	Ph.D.	1969	Immunology

RESEARCH AND/OR PROFESSIONAL EXPERIENCE: Concluding with present position, list in chronological order previous employment, experience, and honors. Key personnel include the principal investigator and any other individuals who participate in the scientific development or execution of the project. Key personnel typically will include all individuals with doctoral or other professional degrees, but in some projects will include individuals at the masters or baccalaureate level provided they contribute in a substantive way to the scientific development or execution of the project. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles, all authors, and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. DO NOT EXCEED TWO PAGES.

Professional Experience:

1969	Postdoctoral Fellow, Yale University, Department of Pathology (R.K. Gershon)
1969-1970	Postdoctoral Fellow, Oxford University, England (J.L. Gowans) *Jane Coffin Childs Fellow*
1971-1974	Assistant Professor of Immunology, Dept. of Microbiology & Immunology, Duke Univ. Medical Center
1974-1979	Associate Professor of Immunology, Duke University Medical Center (tenured)
1975	Visiting Scientist, University of Alberta, Department of Immunology (E. Diener, June-Aug)
1976-1977	Sabbatical Visitor at Walter and Eliza Hall Institute, Melbourne, Australia (G.J.V. Nossal) *Eleanor Roosevelt Fellowship*
1979-1983	Professor, Department of Microbiology & Immunology, Duke University Medical Center
1981	Visiting Scientist, Scripps Clinical & Res. Foundation, La Jolla, CA (N. Klinman, June-July)
1983-1994	Dean's Professor of Immunology and Head, Immunology Division, Cancer Center and Professor of Microbiology and Immunology, University of Rochester, Rochester, NY
1986	Visiting Scientist, National Institute for Medical Research, London, U.K. (G.G.B. Klaus, May-December) *Eleanor Roosevelt Fellowship*
1993	Visiting Scientist, Max-Planck Institute, Freiburg, Germany (G. Köhler, June-August)
1994-	Head, Department of Immunology, American Red Cross, Holland Laboratory, Rockville, MD and Professor of Microbiology and Immunology, George Washington Univ. Medical Center (1998) Director, Immunology Training Program, 1999.
1996-	Adjunct Professor of Microbiology and Immunology, Georgetown Univ. Medical Center

Honors and Awards:

Jane Coffin Childs Postdoctoral Trainee, Oxford, England (1969-70)
Research Career Development Awardee, NIH (1975-80)
Eleanor Roosevelt Fellow, International Union Against Cancer (1976-77; 1986)
Dean's Professor of Immunology, University of Rochester Medical Ctr. (1983-1994)

Professional Activities:

NIH Study Section Member, IMB, 1989, 1995, 1997, 1998; AIDS (ARR-1), 1990; IMS-4, 1995; DAIDS Panel, Innovative Vaccines, 1998.
American Cancer Society Advisory Committee, Immunol. & Immunotherapy, 1982-86, Vice-Chairman, 1984, Chairman, 1985-86. Council Member, Fall 1990
Associate Editor, *Journal of Immunology*, 1980-84; Section Editor, 1993-1997
Associate Editor, *Cancer Immunology and Immunotherapy*, 1984-88
Editorial Board, *Cellular Immunology*, 1994-present
Education Committee, AAI, 1980-85, 1993-present; Chairman, 1984-84
FASEB Education Committee, 1981-90; Chairman 1987-90; IUIS Educat. Comm., 1986-1989
American Society for Microbiology, Chairman, Pre-College Education Committee, 1990-1994
Acting Director, Immunol. Allergic & Immunol. Diseases Pgrm, NIAID, NIH, summer 1988
Board Member. Triangle Coalition for Science and Technology Education, 1994-1997
Advisory Board, Education Development Center, 1994.
Council, Midwinter Conference of Immunologists, 1997-present.

Research projects ongoing or completed (last three years):

RO1 CA55644-06 (Scott, D.W) NIH, NCI
Regulation of B-Lymphoma Growth and Apoptosis
The major goal of this project is to understand the mechanisms of anti-IgM induced murine B-lymphoma growth arrest and apoptosis, especially with respect to initial signaling, myc and cyclin kinase activity.

PO1 CA78794-01 (Scott, D.W.) NIH, NCI (pending)
Signals Regulating Fas-Mediated Apoptosis in B-cell lines
This project is examining the regulation of Fas-mediated apoptosis in a series of murine and human B-cell lines. In particular, we are studying the pathways by which anti-Ig crosslinking renders cells Fas-resistant and its role in lymphomagenesis. A second goal its to understand the pathways on natural resistance to Fas-driven apoptosis in a subset of these lines.

R01 AI35622-01 (Scott, D.W.) NIH, NIAID
Novel Gene Therapy for Tolerance Induction
The aims of these projects are to establish a bone marrow retroviral transmission approach to autoantigenic epitopes and apply this to a model (EAE) for MS.

#196110 (Scott, D.W.) Juvenile Diabetes Foundation International
Novel Gene Therapies for the Induction of Tolerance in Diabetes
The aims of this project are to establish a bone marrow retroviral transmission approach to autoantigenic epitopes and apply this to a model of diabetes.

RO1 AI 29691-10 (Scott, D.W.) NIH, NIAID
Regulation of Specific B-Cell Responsiveness to gp120
The aims of this project are to develop strategies for tolerance to define epitopes in HIV gp120 and apply these to a model of CD4 T-cell apoptosis induced by anti-gp120:gp-120 crosslinking.

Publications (selected from over 170) **David W. Scott, Ph.D.**

Alés-Martinez, J.E., Warner, G.L., and Scott, D.W.: Immunoglobulins D and M mediate signals that are qualitatively different in B cells with an immature phenotype. Proc. Natl. Acad. Sci. USA 85: 6919-6923, 1988.

Yao, X-r. and Scott, D.W.: Expression of protein tyrosine kinases in the Ig complex of anti-μ sensitive and anti-μ resistant B-cell lymphomas: A role of the p55^{blk} kinase in signaling growth arrest and apoptosis. Immunol. Rev., 132: 163-186, 1993.

Scott, D.W. B-cell tolerance in vitro. Advances in Immunology, 54: 393-425, 1993.

Fischer, G., Kent, S., Joseph, L., Green, D.R., and Scott, D.W.: Effect of antisense oncogene oligonucleotides on signal transduction in growth inhibitable murine B-cell lymphomas. J. Exp. Med., 179: 221-228, 1994.

Green, D. R. and Scott, D.W. Activation-induced apoptosis in lymphocytes. Current Opinion in Immunology, 6: 476-487, 1994.

Joseph, L., Ezhevsky, S., and Scott, D.W. Lymphoma models for B-cell activation and tolerance. XI. Anti-IgM treatment induces growth arrest by preventing the formation of an active kinase complex which phosphorylates pRB in G_1. Cell Growth and Differentiation, 6: 51, 1995.

Yao, X-r., Flaswinkel, H.,.Reth, M. and Scott, D.W. Iga or Igb Cytoplasmic tails containing an Immunoreceptor Tyrosine-based Activation Motif (ITAM) can independently signal for growth arrest and apoptosis in murine B-lymphoma cells. J. Immunology 155: 652, 1995.

Ezhevsky, S., Toyoshima, H. , Hunter, T. and Scott, D.W. Role of cyclin A and p27 in anti-IgM-induced G_1 growth arrest of murine B-cell lymphomas, Molecular Biol. Cell, 7: 553 1996.

Zambidis, E., and Scott, D.W. Epitope-specific tolerance induction with an engineered immunoglobulin. Proc. Nat. Acad. Sci. 93: 5019, 1996.

Scott, D.W., Grdina, T. and Shi, Y. T cells commit suicide but B cells are murd red. J. Immunology, 156: 2352, 1996.

Scott, D.W., Lamers, M., Köhler, G., Sidman, C., Maddox, B., Wang, R., and Carsetti, R. Regulation of spontaneous and anti-receptor induced apoptosis in adult murine B-cells by c-Myc. Inter. Immunology, 8: 1375, 1996.

Zambidis, E., Kurup, A. and Scott, D.W. Genetically-transferred central and peripheral immune tolerance via retroviral-mediated expression of immunogenic epitopes in hematopoietic progenitors or peripheral B lymphocytes. Molec. Medicine, 3: 212, 1997.

Zambidis, E., Barth, R. and Scott, D.W. Both resting and activated B lymphocytes expressing engineered peptide-immunoglobulin molecules serve as highly efficient tolerogenic vehicles in immunocompetent adult recipients. J. Immunology, 158: 2174, 1997.

Scott, D.W., Brunner, T., Donjerkovic', D., Ezhevsky, S., Grdina, T., Green, D., Shi, Y. and Yao, X.-R. Murder and suicide: A tale of T and B cell apoptosis. In: Programmed Cell Death: (ed. by Y. B. Shi, Y. Shi, D.W. Scott and X. Yu), Plenum Press, New York, p. 91,1997.

Scott, D.W., Donjerkovic', D., Maddox, B., Ezhevsky, S., and Grdina, T. Role of c-myc and p27 In anti-IgM induced B-lymphoma apoptosis. In: Mechanisms in B-cell neoplasia (ed. by M. Potter and F. Melchers) Contemp. Topics in Immunobiol. p. 103, 1997.

Kang, Y. and Scott, D.W. An ongoing immune response to HIV envelope glycoiprotein in Human CD4 transgenic mice contributes to T cell decline upon intravenous administration of gp120. Eur. J. Immunol. 28: 2253-2264, 1998.

Donjerkovic', D., and Scott, D.W. Regulation of p27kip1 accumulation in murine B-lymphoma cells: role of c-myc and calcium. Cell Growth and Differentiation, 10: 695-704.

Kang, Y., Melo, M., Deng, E., Tisch, R., El-Amine, M. and Scott, D.W. (1999) Induction of hyporesponsiveness to intact multi-determinant foreign protein via retroviral-mediated gene expression: the IgG scaffold is important for induction and maintenance of immunological hyporesponsiveness. Proc. Nat. Acad. Sci., 96: 8609.

Mueller, C.M., and D.W. Scott. 1999. Differential sensitivity of murine B lymphoma cell lines to ligation of the Fas receptor. Submitted. Under revision.

Mueller, C., J.A. Hinshaw, and D.W. Scott. 1999. B-cell receptor-induced protection from Fas-mediated apoptosis. In preparation

Agarwal, R. K., Kang, Y., Zambidis, E., Scott, D.W., Chan, C. and Caspi, R.R. 1996. Retroviral gene transfer of an immunoglobulin-antigen fusion protein protects from autoimmune disease. Submitted.

Scott, D.W., Donjerković, D., Carey, G., Mueller, C., Liu, S., and Tonnetti, L. B-cell receptor and F s-mediated signals for life and death. Immunol. Reviews 176: in press, 2000.

Donjerkovic, D., Carey, G., Mueller, C., Liu, S., and Scott, D.W. Life and death decisions in B1 lymphomas. In: Mechanisms in B-cell neoplasia: Role of B1 and natural antibody producing cells in B-cell neoplasia (ed. by M. Potter and F. Melchers) Contemp. Topics in Immunobiol. In press, 2000.

EXHIBIT B

Figure 1

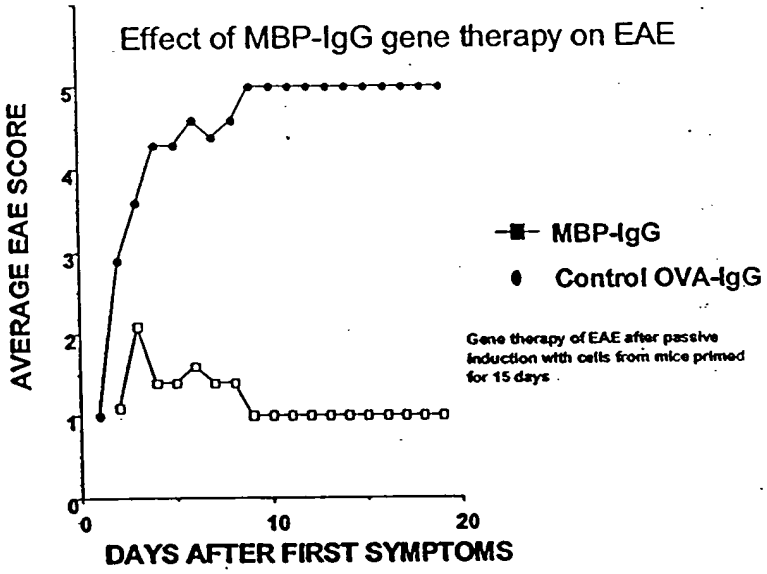
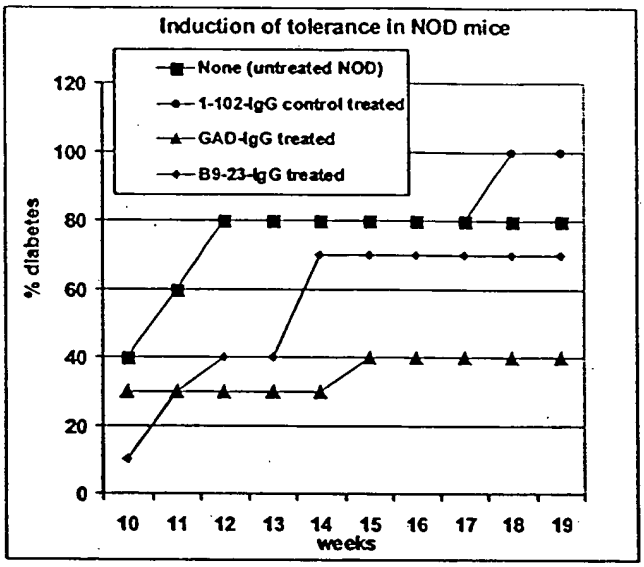

Effect of MBP-IgG gene therapy on EAE
AVERAGE EAE SCORE
0
1
2
3
4
5
0
10
20
DAYS AFTER FIRST SYMPTOMS
MBP-IgG
Control OVA-IgG
Gene therapy of EAE after passive induction with cells from mice primed for 15 days

EXHIBIT C

Figure 2


Induction of tolerance in NOD mice
None (untreated NOD)
1-102-IgG control treated
GAD-IgG treated
B9-23-IgG treated
% diabetes
120
100
80
60
40
20
0
10
11
12
13
14
15
16
17
18
19
weeks